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      (FILE 'HOME' ENTERED AT 09:54:16 ON 13 JAN 2003)

      FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS, USPATFULL' ENTERED AT 09:54:36
      ON 13 JAN 2003
L1            15 S (MIMITOPE? OR (ANALYTE ANALOG?)) (6P) (DISPLACEMENT ASSAY?)
L2            15 DUP REM L1 (0 DUPLICATES REMOVED)
L3          1685 S (DISPLACEMENT ASSAY?)
L4             0 S L3/AB AND (MIMITOPE? OR (ANALYTE ANALOG?))
L5           727 S (DISPLACEMENT ASSAY?)/AB
L6             0 S L5 (10P) (MIMITOPE? OR (ANALYTE ANALOG?))
L7             0 S L5 AND (MIMITOPE? OR (ANALYTE ANALOG?))
L8            56 S L3 AND (MIMITOPE? OR (ANALYTE ANALOG?))
L9            56 DUP REM L8 (0 DUPLICATES REMOVED)
L10            5 S L3 AND (MIMITOPE?)
L11            5 DUP REM L10 (0 DUPLICATES REMOVED)
L12           92 S ((FUSION PROTEIN?) OR CHIMER?) (6P) (DISPLACEMENT ASSAY?)
L13           43 S ((FUSION PROTEIN?) OR CHIMER?) (P) (DISPLACEMENT ASSAY?)
L14           26 DUP REM L13 (17 DUPLICATES REMOVED)

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09/554,958

L2 ANSWER 10 OF 15 USPATFULL
ACCESSION NUMBER: 1998:134508 USPATFULL
TITLE: Electrodes and metallo isoindole ringed compounds
INVENTOR(S): Gilmartin, Markas A. T., 18 Westbrook Court, Cumberland Close, Baltic Wharf Bristol BS16XB, England

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 5830341		19981103	
APPLICATION INFO.:	US 1996-589106		19960123	(8)

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Gorgos, Kathryn L.
ASSISTANT EXAMINER: Noguerola, Alex
LEGAL REPRESENTATIVE: Cooley Godward LLP
NUMBER OF CLAIMS: 40
EXEMPLARY CLAIM: 32
NUMBER OF DRAWINGS: 8 Drawing Figure(s); 5 Drawing Page(s)
LINE COUNT: 1682
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides for electrode assemblies and methods in which electron transfer between a redox reaction product and an electrically conductive electrode material is facilitated by a metallo macrocyclic compound, preferably a metallo isoindole ringed compound and more preferably a ferro isoindole ringed compound. The redox reaction is usually catalysized by a redox enzyme, such as an oxidase.

DETD The enzyme layer can also be comprised of a first enzyme attached to an analyte or **analyte analog**. Preferably, an oxidase is attached to an analyte or an **analyte analog**. The enzyme attached to the analyte or **analyte analog** can prevent the analyte from binding to an analyte binding moiety. Such enzyme layers can be in assays where the. . . is attached to a matrix in the enzyme layer. Enzyme layers with a first enzyme attached to an analyte or **analyte analog** are particularly useful for competition or **displacement assays** of the analytes. In such assays, the assay is usually designed to measure a decrease the current in the presence. . .

DETD . . . For example, the contacting step can comprise 1) contacting the analyte with an analyte attached to an oxidase or an **analyte analog** attached to the oxidase. The analyte and either 1) the analyte attached to the oxidase or 2) the **analyte analog** attached to the oxidase are recognized by an analyte binding moiety. Such methods can be applicable to competitive or **displacement assays**. Such methods can be suitable combined with other methods and devices taught herein and known in the art.

2 ANSWER 13 OF 15 USPATFULL

ACCESSION NUMBER: 96:24872 USPATFULL

TITLE: Analyte-substitute reagent for use in specific binding assay methods, devices and kits

INVENTOR(S): Baugher, Bennett W., Waukegan, IL, United States
Chamberlain, Aurora J., Buffalo Grove, IL, United States
Devereaux, Sharon M., Gurnee, IL, United States
Ungemach, Frank S., Lake Villa, IL, United States

PATENT ASSIGNEE(S): Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5501985		19960326
APPLICATION INFO.:	US 1994-230995		19940421 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1993-67254, filed on 25 May 1993, now patented, Pat. No. US 5340748 which is a continuation of Ser. No. US 1990-554304, filed on 18 Jul 1990, now abandoned

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Scheiner, Toni R.
ASSISTANT EXAMINER: Wolski, Susan C.
LEGAL REPRESENTATIVE: Steele, Gregory W.
NUMBER OF CLAIMS: 4
EXEMPLARY CLAIM: 1
LINE COUNT: 1315
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Assay reagents, devices, methods and kits used in the analysis of low molecular weight analytes which by themselves are too small or unable to bind to two specific binding members at the same time. The invention involves the use of an analyte-substitute reagent (ASR) comprising at least two components, the first of which is identical to or an analog of the analyte to be determined, while the second is an unrelated ligand for which an antibody or other specific binding member can be obtained or produced.

SUMM . . . a capture reagent or wherein the analyte displaces the indicator reagent from the capture reagent. A positive result in a **displacement assay** is indicated by a decrease in the amount of label associated with the solid phase material. Visually determining a positive. . .

SUMM The ASR comprises a first component which is an analyte, **analyte -analog** or other ligand having at least one epitope in common with the analyte, thereby enabling the ASR to bind to. . .

L9 ANSWER 30 OF 56 USPATFULL
ACCESSION NUMBER: 2001:163060 USPATFULL
TITLE: Specific binding assays
INVENTOR(S): Badley, Robert A., Bedford, United Kingdom
Berry, Mark J., Northampton, United Kingdom
Porter, Philip, Bedford, United Kingdom
Wattam, Trevor A., Royston, United Kingdom
PATENT ASSIGNEE(S): Unilever Patent Holdings B.V., AT Vlaardingen, Netherlands (non-U.S. corporation)

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6294391	B1	20010925	
APPLICATION INFO.:	US 1997-861693		19970522	(8)

	NUMBER	DATE
PRIORITY INFORMATION:	EP 1996-303693	19960523

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Chin, Christopher L.
LEGAL REPRESENTATIVE: Pillsbury Winthrop LLP
NUMBER OF CLAIMS: 8
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 6 Drawing Page(s)
LINE COUNT: 809
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed is a method of detecting the presence of an analyte of interest in a sample, the method comprising the steps of:

providing a binding partner reversibly immobilised on a solid support, said binding partner having binding specificity for the analyte;

contacting the sample with the solid support;

specifically displacing the binding partner from the solid support in response to the presence of the analyte of interest in the sample, said displacement causing a reduction in the mass of material immobilised on the solid support, thereby generating a detectable change in a mass-dependent property of the solid support; and

detecting said change. Also disclosed is an assay device for performing the method of the invention.

SUMM A refinement of assays of the general nature outlined above relates to "**displacement" assays**. In such assays, the presence of an analyte of interest in a sample causes the displacement either of a labelled. . .

SUMM . . . of a number of ways, which will be apparent to the person skilled in the art. The binding partner or **analyte analogue** may normally be removed from the solid support by application of particular chemicals (e.g. solutions, such as 50 mM glycine,. . . the assay conditions will be broadly physiological (e.g. about 10-40.degree. C., about pH 5-9), such that the binding partner or **analyte analogue** will only be released from the solid support by the presence of the analyte of interest.

SUMM . . . such that one binding site interacts with antigen bound to a solid support, leaving another free to be occupied by **analyte analogue** prior to assay. Alternatively, the antibody could possess a single binding site (like Fv or Fab fragments of Ig) but. . .

L14 ANSWER 12 OF 26 USPATFULL

ACCESSION NUMBER: 2000:21421 USPATFULL
TITLE: Human trk receptors and neurotrophic factor inhibitors
INVENTOR(S): Presta, Leonard G., San Francisco, CA, United States
Shelton, David L., Pacifica, CA, United States
Urfer, Roman, Pacifica, CA, United States
PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6027927		20000222
APPLICATION INFO.:	US 1997-942562		19971001 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1995-444597, filed on 19 May 1995, now abandoned which is a continuation-in-part of Ser. No. US 1994-286846, filed on 5 Aug 1994, now patented, Pat. No. US 5877016 which is a continuation of Ser. No. US 1994-215139, filed on 18 Mar 1994, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Teng, Sally
LEGAL REPRESENTATIVE: Torchia, Timothy E., Johnston, Sean A.
NUMBER OF CLAIMS: 17
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 24 Drawing Figure(s); 26 Drawing Page(s)
LINE COUNT: 4565
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns human trkB and trkC receptors and their functional derivatives. The invention further concerns immunoadhesins comprising trk receptor sequences fused to immunoglobin sequences.

DETD In order to test whether these **chimeric** proteins retained the binding specificity expected of the trk extracellular domain in a cellular environment, competitive **displacement assays** were done with iodinated neurotrophins. As can be seen from the results shown in FIG. 10, the trk-IgG **chimeras** did show specific binding to the expected neurotrophin(s). **Chimeras** containing trkA extracellular domain bound NGF well and NT3 and NT5 with much lower affinity. **Chimeras** containing trkB bound BDNF and NT5 well but only slightly better than NT3, and showed almost no detectable binding to NGF. **Chimeras** containing trkC were highly specific for NT3 over the other neurotrophins. The apparent affinity of the **chimeras** for their preferred ligand as determined in these competitive **displacement assays** is in the range of that determined for the majority of the binding sites on cells transfected with and expressing. . .

L14 ANSWER 13 OF 26 USPATFULL

ACCESSION NUMBER: 2000:18252 USPATFULL
TITLE: Human trk receptors and neurotrophic factor inhibitors
INVENTOR(S): Presta, Leonard G., San Francisco, CA, United States
Shelton, David L., Pacifica, CA, United States
Urfer, Roman, Pacifica, CA, United States
PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6025166		20000215
APPLICATION INFO.:	US 1995-444622		19950519 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-286846, filed on 5 Aug 1994, now patented, Pat. No. US 5877016 which is a continuation-in-part of Ser. No. US 1994-215139, filed on 18 Mar 1994, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Hutzell, Paula K.
ASSISTANT EXAMINER: Davis, Minh-Tam
LEGAL REPRESENTATIVE: Torchia, Timothy E.
NUMBER OF CLAIMS: 33
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 36 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 4660
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns human trkB and trkC receptors and their functional derivatives. The invention further concerns imnunoadhesins comprising trk receptor sequences fused to immunoglobulin sequences.

DETD In order to test whether these **chimeric** proteins retained the binding specificity expected of the trk extracellular domain in a cellular environment, competitive **displacement assays** were done with iodinated neurotrophins. As can be seen from the results shown in FIG. 10, the trk-IgG **chimeras** did show specific binding to the expected neurotrophin(s). **Chimeras** containing trkA extracellular domain bound NGF well and NT3 and NT5 with much lower affinity. **Chimeras** containing trkB bound BDNF and NT5 well but only slightly better than NT3, and showed almost no detectable binding to NGF. **Chimeras** containing trkC were highly specific for NT3 over the other neurotrophins. The apparent affinity of the **chimeras** for their preferred ligand as determined in these competitive **displacement assays** is in the range of that determined for the majority of the binding sites on cells transfected with and expressing. . .

L14 ANSWER 15 OF 26 USPATFULL

ACCESSION NUMBER: 1999:65330 USPATFULL

TITLE: Human trk receptors and neurotrophic factor inhibitors

INVENTOR(S): Presta, Leonard G., San Francisco, CA, United States
Shelton, David L., Pacifica, CA, United States
Urfer, Roman, Pacifica, CA, United States

PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5910574		19990608
APPLICATION INFO.:	US 1995-457880		19950531 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-286846, filed on 5 Aug 1994 which is a continuation-in-part of Ser. No. US 1994-215139, filed on 18 Mar 1994, now abandoned

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted

PRIMARY EXAMINER: Huff, Sheela

ASSISTANT EXAMINER: Reeves, Julie E

LEGAL REPRESENTATIVE: Torchia, Timothy E.

NUMBER OF CLAIMS: 15

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 47 Drawing Figure(s); 28 Drawing Page(s)

LINE COUNT: 4244

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns human trkB and trkC receptors and their functional derivatives. The invention further concerns immunoadhesins comprising trk receptor sequences fused to immunoglobulin sequences.

DETD In order to test whether these **chimeric** proteins retained the binding specificity expected of the trk extracellular domain in a cellular environment, competitive **displacement assays** were done with iodinated neurotrophins. As can be seen from the results shown in FIG. 10, the trk-IgG **chimeras** did show specific binding to the expected neurotrophin(s). **Chimeras** containing trkA extracellular domain bound NGF well and NT3 and NT5 with much lower affinity. **Chimeras** containing trkB bound BDNF and NTS well but only slightly better than NT3, and showed almost no detectable binding to NGF. **Chimeras** containing trkC were highly specific for NT3 over the other neurotrophins. The apparent affinity of the **chimeras** for their preferred ligand as determined in these competitive **displacement assays** is in the range of that determined for the majority of the binding sites on cells transfected with and expressing. . .

L14 ANSWER 13 OF 26 USPATFULL

ACCESSION NUMBER: 2000:18252 USPATFULL
TITLE: Human trk receptors and neurotrophic factor inhibitors
INVENTOR(S): Presta, Leonard G., San Francisco, CA, United States
Shelton, David L., Pacifica, CA, United States
Urfer, Roman, Pacifica, CA, United States
PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6025166		20000215
APPLICATION INFO.:	US 1995-444622		19950519 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-286846, filed on 5 Aug 1994, now patented, Pat. No. US 5877016 which is a continuation-in-part of Ser. No. US 1994-215139, filed on 18 Mar 1994, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Hutzell, Paula K.
ASSISTANT EXAMINER: Davis, Minh-Tam
LEGAL REPRESENTATIVE: Torchia, Timothy E.
NUMBER OF CLAIMS: 33
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 36 Drawing Figure(s); 15 Drawing Page(s)
LINE COUNT: 4660
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns human trkB and trkC receptors and their functional derivatives. The invention further concerns imnunoadhesins comprising trk receptor sequences fused to immunoglobulin sequences.

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performed at room temperature. . .

L14 ANSWER 15 OF 26 USPATFULL
ACCESSION NUMBER: 1999:65330 USPATFULL
TITLE: Human trk receptors and neurotrophic factor inhibitors
INVENTOR(S): Presta, Leonard G., San Francisco, CA, United States
Shelton, David L., Pacifica, CA, United States
Urfer, Roman, Pacifica, CA, United States
PATENT ASSIGNEE(S): Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5910574		19990608
APPLICATION INFO.:	US 1995-457880		19950531 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1994-286846, filed on 5 Aug 1994 which is a continuation-in-part of Ser. No. US 1994-215139, filed on 18 Mar 1994, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Huff, Sheela
ASSISTANT EXAMINER: Reeves, Julie E
LEGAL REPRESENTATIVE: Torchia, Timothy E.
NUMBER OF CLAIMS: 15
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 47 Drawing Figure(s); 28 Drawing Page(s)
LINE COUNT: 4244
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns human trkB and trkC receptors and their functional derivatives. The invention further concerns immunoadhesins comprising trk receptor sequences fused to immunoglobulin sequences.

DETD In order to test whether these **chimeric** proteins retained the binding specificity expected of the trk extracellular domain in a cellular environment, competitive **displacement assays** were done with iodinated neurotrophins. As can be seen from the results shown in FIG. 10, the trk-IgG **chimeras** did show specific binding to the expected neurotrophin(s). **Chimeras** containing trkA extracellular domain bound NGF well and NT3 and NT5 with much lower affinity. **Chimeras** containing trkB bound BDNF and NTS well but only slightly better than NT3, and showed almost no detectable binding to NGF. **Chimeras** containing trkC were highly specific for NT3 over the other neurotrophins. The apparent affinity of the **chimeras** for their preferred ligand as determined in these competitive **displacement assays** is in the range of that determined for the majority of the binding sites on cells transfected with and expressing. . .

L14 ANSWER 26 OF 26 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 7
ACCESSION NUMBER: 92:105137 SCISEARCH
THE GENUINE ARTICLE: HD182
TITLE: PROPERTIES OF CHIMERIC TOXINS WITH 2 RECOGNITION DOMAINS - INTERLEUKIN 6 AND TRANSFORMING GROWTH-FACTOR ALPHA AT DIFFERENT LOCATIONS IN PSEUDOMONAS EXOTOXIN
AUTHOR: KREITMAN R J; SIEGALL C B; CHAUDHARY V K; FITZGERALD D J; PASTAN I (Reprint)
CORPORATE SOURCE: NCI, DCBDC, MOLEC BIOL LAB, BETHESDA, MD, 20892 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: BIOCONJUGATE CHEMISTRY, (JAN/FEB 1992) Vol. 3, No. 1, pp. 63-68.
ISSN: 1043-1802.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 22

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pseudomonas exotoxin (PE) is a potent cytotoxic agent that is composed of 613 amino acids arranged into three major domains. We have previously identified two positions where ligands can successfully be placed in PE to direct it to cells with specific surface receptors. One site is at the amino terminus and the other is close to but not at the C-terminus. To examine the possibility of constructing oncotoxins with two different recognition elements that will bind to two different receptors, we have placed cDNAs encoding either transforming growth factor-alpha (TGF-alpha) or interleukin 6 (IL6) at the 5' end of a PE gene and also inserted a cDNA encoding TGF-alpha near the 3' end of the PE gene. The plasmids encoding these **chimeric** toxins were expressed in Escherichia coli and the **chimeric** proteins purified to near homogeneity. In all the new toxins, the TGF-alpha near the C-terminus was inserted after amino acid 607 of PE and followed by amino acids 604-613 so that the correct PE C-terminus (REDLK) was preserved. For each **chimera**, the toxin portion was either PE4E, in which the cell binding domain (domain Ia) is mutated, PE40, in which domain la is deleted, or PE38, in which domain la and part of domain Ib are deleted. These derivatives of PE do not bind to the PE receptor and allow 607, 355, or 339 amino acids, respectively, between the two ligands. **Chimeric** toxins containing two TGF-alpha ligands were all cytotoxic to human cancer cells expressing EGF receptors, while those containing one IL6 and one TGF-alpha ligand were cytotoxic toward cells expressing either IL6 or EGF receptors, or both. The effect of distance separating the two ligands was evaluated using cytotoxicity assays and [I-125]EGF **displacement assays** . The animal toxicity of two of the bifunctional **chimeric** toxins was investigated in mice. Our results establish that two ligands can be placed in different locations within PE simultaneously and that adding IL6 to the amino terminus of PE which already contains TGF-alpha near the carboxyl terminus decreases animal toxicity in vivo and yet increases cytotoxicity against some cell lines in vitro.

L14 ANSWER 26 OF 26 SCISEARCH COPYRIGHT 2003 ISI (R) DUPLICATE 7
ACCESSION NUMBER: 92:105137 SCISEARCH
THE GENUINE ARTICLE: HD182
TITLE: PROPERTIES OF CHIMERIC TOXINS WITH 2 RECOGNITION DOMAINS - INTERLEUKIN 6 AND TRANSFORMING GROWTH-FACTOR ALPHA AT DIFFERENT LOCATIONS IN PSEUDOMONAS EXOTOXIN
AUTHOR: KREITMAN R J; SIEGALL C B; CHAUDHARY V K; FITZGERALD D J; PASTAN I (Reprint)
CORPORATE SOURCE: NCI, DCBDC, MOLEC BIOL LAB, BETHESDA, MD, 20892 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: BIOCONJUGATE CHEMISTRY, (JAN/FEB 1992) Vol. 3, No. 1, pp. 63-68.
ISSN: 1043-1802.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 22

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Pseudomonas exotoxin (PE) is a potent cytotoxic agent that is composed of 613 amino acids arranged into three major domains. We have previously identified two positions where ligands can successfully be placed in PE to direct it to cells with specific surface receptors. One site is at the amino terminus and the other is close to but not at the C-terminus. To examine the possibility of constructing oncotoxins with two different recognition elements that will bind to two different receptors, we have placed cDNAs encoding either transforming growth factor-alpha (TGF-alpha) or interleukin 6 (IL6) at the 5' end of a PE gene and also inserted a cDNA encoding TGF-alpha near the 3' end of the PE gene. The plasmids encoding these **chimeric** toxins were expressed in Escherichia coli and the **chimeric** proteins purified to near homogeneity. In all the new toxins, the TGF-alpha near the C-terminus was inserted after amino acid 607 of PE and followed by amino acids 604-613 so that the correct PE C-terminus (REDLK) was preserved. For each **chimera**, the toxin portion was either PE4E, in which the cell binding domain (domain Ia) is mutated, PE40, in which domain la is deleted, or PE38, in which domain la and part of domain Ib are deleted. These derivatives of PE do not bind to the PE receptor and allow 607, 355, or 339 amino acids, respectively, between the two ligands. **Chimeric** toxins containing two TGF-alpha ligands were all cytotoxic to human cancer cells expressing EGF receptors, while those containing one IL6 and one TGF-alpha ligand were cytotoxic toward cells expressing either IL6 or EGF receptors, or both. The effect of distance separating the two ligands was evaluated using cytotoxicity assays and [I-125]EGF **displacement assays** . The animal toxicity of two of the bifunctional **chimeric** toxins was investigated in mice. Our results establish that two ligands can be placed in different locations within PE simultaneously and that adding IL6 to the amino terminus of PE which already contains TGF-alpha near the carboxyl terminus decreases animal toxicity in vivo and yet increases cytotoxicity against some cell lines in vitro.

L14 ANSWER 23 OF 26 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 95156440 MEDLINE
DOCUMENT NUMBER: 95156440 PubMed ID: 7853357
TITLE: Characterization of heterologously expressed recombinant retinoic acid receptors with natural or synthetic retinoids.
AUTHOR: Kim Y W; Sharma R P; Li J K
CORPORATE SOURCE: Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan 84322.
CONTRACT NUMBER: HD 28259 (NICHD)
SOURCE: JOURNAL OF BIOCHEMICAL TOXICOLOGY, (1994 Oct) 9 (5) 225-34. Journal code: 8700114. ISSN: 0887-2082.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 19980206
Entered Medline: 19950310

AB The first step in retinoid action is binding to their nuclear receptors. Therefore, characterization of binding characteristics of retinoids is of major importance. Human retinoic acid receptors alpha (hRAR alpha), hRAR beta, and mouse RAR gamma (mRAR gamma) were expressed heterologously in Escherichia coli as a recombinant glutathione S-transferase (GST) **fusion protein**. The expressed **fusion proteins** were functional and bound specifically to the all-trans-retinoic acid (RA). The dissociation constants (Kd) for RA were 1.4 nM for GST-hRAR alpha, 1.4 nM for GST-hRAR beta, and 3.3 nM for GST-mRAR gamma, respectively. The **fusion proteins** were further used for competitive **displacement assays** to determine the displacement constant (DC50) for other selected retinoids. All-trans-RA and 4-oxo-all-trans-RA have high affinity with all three receptors (DC50 = 0.8-55 nM). The 13-cis RA binds to hRAR alpha with low affinity, but not to other RARs evaluated here. All-trans-N-ethylretinamide, all-trans-retinylacetate, and an ethyl ester of tetrahydronaphthalene derivative had no affinity to any RARs. The hRAR alpha and mRAR gamma receptors did not bind a naphthalene carboxylic acid derivative of RA, but hRAR beta binds this chemical with high affinity. Results indicated that the three recombinant proteins were functional in binding various RA congeners. The affinity and binding data of these retinoids were compared to their observed teratogenic activity.

L14 ANSWER 23 OF 26 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 95156440 MEDLINE
DOCUMENT NUMBER: 95156440 PubMed ID: 7853357
TITLE: Characterization of heterologously expressed recombinant retinoic acid receptors with natural or synthetic retinoids.
AUTHOR: Kim Y W; Sharma R P; Li J K
CORPORATE SOURCE: Department of Animal, Dairy and Veterinary Sciences, Utah State University, Logan 84322.
CONTRACT NUMBER: HD 28259 (NICHD)
SOURCE: JOURNAL OF BIOCHEMICAL TOXICOLOGY, (1994 Oct) 9 (5) 225-34. Journal code: 8700114. ISSN: 0887-2082.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199503
ENTRY DATE: Entered STN: 19950322
Last Updated on STN: 19980206
Entered Medline: 19950310

AB The first step in retinoid action is binding to their nuclear receptors. Therefore, characterization of binding characteristics of retinoids is of major importance. Human retinoic acid receptors alpha (hRAR alpha), hRAR beta, and mouse RAR gamma (mRAR gamma) were expressed heterologously in Escherichia coli as a recombinant glutathione S-transferase (GST) **fusion protein**. The expressed **fusion proteins** were functional and bound specifically to the all-trans-retinoic acid (RA). The dissociation constants (Kd) for RA were 1.4 nM for GST-hRAR alpha, 1.4 nM for GST-hRAR beta, and 3.3 nM for GST-mRAR gamma, respectively. The **fusion proteins** were further used for competitive **displacement assays** to determine the displacement constant (DC50) for other selected retinoids. All-trans-RA and 4-oxo-all-trans-RA have high affinity with all three receptors (DC50 = 0.8-55 nM). The 13-cis RA binds to hRAR alpha with low affinity, but not to other RARs evaluated here. All-trans-N-ethylretinamide, all-trans-retinylacetate, and an ethyl ester of tetrahydronaphthalene derivative had no affinity to any RARs. The hRAR alpha and mRAR gamma receptors did not bind a naphthalene carboxylic acid derivative of RA, but hRAR beta binds this chemical with high affinity. Results indicated that the three recombinant proteins were functional in binding various RA congeners. The affinity and binding data of these retinoids were compared to their observed teratogenic activity.

L8 ANSWER 5 OF 10 USPATFULL
ACCESSION NUMBER: 96:24872 USPATFULL
TITLE: Analyte-substitute reagent for use in specific binding assay methods, devices and kits
INVENTOR(S): Baugher, Bennett W., Waukegan, IL, United States
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Assay reagents, devices, methods and kits used in the analysis of low molecular weight analytes which by themselves are too small or unable to bind to two specific binding members at the same time. The invention involves the use of an analyte-substitute reagent (ASR) comprising at least two components, the first of which is identical to or an analog of the analyte to be determined, while the second is an unrelated ligand for which an antibody or other specific binding member can be obtained or produced.

SUMM Various analytical procedures and devices are commonly employed in **assays** to determine the presence and/or concentration of substances of interest or clinical significance which may be present in biological fluids. . . on the ability of health care professionals to treat and manage a pathological disorder. In addition, the performance of such **assays** enables early and accurate determination of physiological conditions such as pregnancy, the monitoring of drug therapy and the evaluation of. . .

SUMM Typical **immunoassay** techniques utilize the mechanisms of the immune systems of higher organisms, wherein antibodies are produced in response to the presence. . .

SUMM Heterogeneous **immunoassay** techniques typically involve the use of a **solid** phase material to which the reaction product becomes bound. The reaction product is separated from excess sample, **assay** reagents and other substances by removing the **solid** phase from the reaction mixture. One type of **solid** phase **immunoassay** is a sandwich **immunoassay** involving an anti-analyte antibody (**capture** reagent) bound to the insoluble **solid** phase material, as described by Schuurs et al. U.S. Pat. Nos. 3,791,932 and 4,016,043. A second anti-analyte antibody is labeled. . . antibodies form an immunocomplex with the analyte (i.e., an antibody/analyte/antibody sandwich), and the amount of indicator reagent associated with the **solid** phase is directly proportional to the amount of analyte in the test sample. When the enzyme substrate is added, it. . . reacts with the enzyme component of the indicator reagent to signal the presence or amount of analyte

associated with the **solid** phase.

SUMM Another type of **solid** phase **immunoassay** configuration is the competitive **assay**. In a manner similar to the sandwich **assay**, the competitive **assay** can involve an anti-**analyte** antibody bound to the insoluble **solid** phase, but a labeled **analyte**, instead of a labeled second antibody, may be used as the indicator reagent. In the competitive **assay**, the indicator reagent competes with the test sample **analyte** to bind the **capture** reagent on the **solid** phase. The amount of **captured** indicator reagent is inversely proportional to the amount of **analyte** present in the test sample. Smith (U.S. Pat. No. 4,401,764) describes an alternative competitive **assay** format using a mixed binding complex which can bind **analyte** or labeled **analyte** but wherein the **analyte** and labeled **analyte** can not simultaneously bind the complex. Clagett (U.S. Pat. No. 4,746,631) describes an **immunoassay** method using a reaction chamber in which an **analyte**/ligand/marker conjugate is **displaced** from the reaction **surface** in the presence of test sample **analyte** and in which the **displaced analyte** /ligand/marker conjugate is immobilized at a second reaction site. The conjugate includes biotin, bovine serum albumin and synthetic peptides as the. . . materials, enzyme inhibitors and radionucleotides as the marker component of the conjugate. Li (U.S. Pat. No. 4,661,444) describes a competitive **immunoassay** using a conjugate of an anti-idiotype antibody and a second antibody, specific for a detectable label, wherein the detectable response is inversely related to the presence of **analyte** in the sample.

SUMM In both the sandwich and competitive **immunoassays**, the presence or amount of analyte in the test sample is generally determined by detecting the presence or amount of the label which has become associated with the **solid** phase. In the competitive **assay**, the more analyte present in the test sample the lower the amount of label present on the **solid** phase. In the sandwich **assay**, the more analyte present in the sample the greater the amount of label present on the **solid** phase. The sandwich **assay** is generally preferred, especially for the visualization of low analyte concentrations, because the appearance of label on the **solid** phase is more readily detected.

SUMM The sandwich **assay**, however, is subject to several limiting factors. Certain analytes of interest, such as some steroids, hormones, antibiotics and other therapeutic. . . two antibodies to the analyte and thereby form the sandwich complex. Methods for detecting such analytes typically use a competitive **assay** configuration wherein the analyte either competes with an indicator reagent for binding to a **capture** reagent or wherein the analyte **displaces** the indicator reagent from the **capture** reagent. A positive result in a **displacement assay** is indicated by a decrease in the amount of label associated with the **solid** phase material. Visually determining a positive **assay** result by detecting a decreasing amount of label is more difficult than detecting the appearance of label on the **solid** phase, and difficulty in discerning a decrease in the amount of label can lead to ambiguity in the interpretation of the **assay** result. Allen (EP 177,191) describes a binding **assay** involving a conjugate of a ligand analog and a second reagent, such as fluorescein, wherein the conjugate competes with the. . . specific for the ligand, and wherein the resultant labeled conjugate is then separated from the reaction mixture by means of **solid** phase carrying a binding partner for the second reagent. This binding **assay** format combines the use of a competitive binding technique and a reverse sandwich **assay** configuration, i.e., the binding of conjugate to the labeled binding member prior to separating conjugate from the mixture by the binding of the conjugate to the **solid**

phase. The **assay** result, however, is determined as in a conventional competitive **assay** wherein the amount of label bound to the **solid** phase is inversely proportional to the amount of analyte in the test sample. Chieregatt et al. (GB 2,084,317) describe a similar **assay** format using an indirectly labeled binding partner specific for the analyte. Mochida et al. (U.S. Pat. No. 4,185,084) also describe. . . to an immobilized antibody and which is then labeled; this method also results in the detection of label on a **solid** phase wherein the amount of label is inversely proportional to the amount of analyte in the test sample. Sadeh et al. (U.S. Pat. No. 4,243,749) describe a similar enzyme **immunoassay** wherein a hapten conjugate competes with analyte for binding to an antibody immobilized upon a **solid** phase.

SUMM The present invention provides an analyte-substitute reagent (ASR), **assay** methods, devices and test kits for performing binding **assays** which are especially useful for determining the presence or amount of analytes of small molecular size. The ASR is capable of reacting with suitable **assay** reagent binding members and thereby form detectable ASR complexes or free ASR in amounts proportional to the amount of analyte. . .

SUMM . . . for the analyte in the formation of a detectable binding member complex, wherein the presence of detectable complex on a **solid** phase is directly proportional to the presence of analyte in the test sample. Such an advantageous direct result is achieved. . . the test sample. The uncomplexed ASR then serves as a substitute for analyte in the formation of a detectable sandwich **assay** complex.

SUMM . . . of binding to an epitope which the test sample analyte and the analyte-component of the ASR have in common; a **capture** reagent comprising a second specific binding member which is specific for the ligand-component of the ASR; and an indicator reagent. . . member specific for the analyte-component of the ASR and a label capable of producing a detectable signal. In such an **assay**, the first and third specific binding members may be the same. Alternatively, the second specific binding member of the **capture** reagent can be specific for the analyte-component of the ASR and the third specific binding member of the indicator reagent can be specific for the ligand-component of the ASR. The test sample can be contacted to the **assay** reagents sequentially, singly or in combination. The **assay** method can also include contacting the label of the indicator reagent with at least one additional signal generating substance which. . .

SUMM In the **assay**, the ASR and the analyte of the test sample compete in binding to the first specific binding member, with the free or unbound ASR serving to complete the formation of a sandwich complex with the **capture** and indicator reagents. The label associated with the complex, or the amount of indicator reagent that is not associated with. . .

SUMM . . . as a tablet, capsule, powder or liquid reagent configuration to which the test sample is added; device embodiments wherein the **capture** reagent is immobilized on a **solid** phase material so that the resultant sandwich complex is immobilized on the **solid** phase material; embodiments wherein the **assay** method includes the step of immobilizing the **capture** reagent on a **solid** phase material; embodiments wherein the **assay** method includes the addition of at least one ancillary specific binding member to complete the **assay** reaction, the sandwich complex or the immobilization of the **capture** reagent; and embodiments wherein multiple analytes are **assayed** on the **solid** phase material using multiple ASRs. Typically, the immobilized **capture** reagent serves as the detection site for the sandwich complex in **assay** devices. Devices are contemplated, however, wherein the detection of the indicator reagent takes place at a site other than or in addition to the immobilized **capture** reagent site.

SUMM A further variation of the present invention involves the use of a preformed binding complex comprising the ASR, a **capture** reagent and an indicator reagent. With the addition of the test sample, the analyte of the test sample is believed to replace the ASR in indicator reagent binding, resulting in the detectable **displacement** of the indicator reagent from the complex.

SUMM . . . of an analyte in a test sample. The kits comprise an ASR and other reagents required for the desired binding **assay**. Furthermore, the present invention includes **assay** devices, especially those allowing the production of self-performing **assays**, i.e., the **solid** phase can include a sufficient number of zones or layers to contain the **assay** reagents in a configuration whereby the **assay** is substantially self-performing upon the addition of test sample.

SUMM . . . indicator reagent either during production of the indicator reagent or prior to the use of the indicator reagent in an **assay** . The addition of a surfactant significantly improves the performance of the indicator reagent and can even revive indicator reagents that. . .

DETD . . . the test sample. The ASR provides at least two binding sites so that at least two additional binding members, e.g., **capture** reagent and indicator reagent, can bind to the ASR to form an immunocomplex that is indicative of the presence or. . .

DETD The problem encountered by conventional **assays** for many of these low molecular weight analytes is that the analytes are too small to allow the binding of two binding members to an analyte at the same time. The sandwich **assay** format requires the presence of at least two binding sites or epitopes on the analyte molecule, with sufficient distance between. . .

DETD . . . invention, the first component of the ASR is an analyte or analyte-analog, having at least one epitope in-common with the **assay**'s analyte of interest. For example, the analyte-analog can be derived from the corresponding analyte by the removal of a reactive. . .

DETD . . . ligand component of the ASR can be virtually any specific binding member as discussed above, which does not interfere with **assay** reagent and analyte binding. Thus, the ligand component could be another antigen, an antibody, a peptide sequence, a nucleotide sequence,. . .

DETD In an **assay** device or kit, the ASR can initially be supplied as a reagent that is reversibly joined to a first specific binding member, wherein the test sample analyte **displaces** the ASR due to the analyte's competitive binding to the first specific binding member. Typically, both the ASR and the first specific binding member are present without being coupled or bound prior to the **assay** procedure. The ASR/first specific binding member complex or mixture can have a variety of configurations, such as a lyophilized powder,. . .

DETD DIAGNOSTIC **ASSAYS**

DETD The ASR can be used in a variety of binding **assay** configurations, and the following methods for using the present invention are intended to be descriptive but not limitative of the invention. Typically, the **assays** are "direct" **assays** in that the specific binding members of the indicator and **capture** reagents directly react with the ASR to form a binding complex. "indirect" **assays** are also contemplated using the present invention, for example, an **assay** wherein the specific binding member of the indicator is specific for an ancillary specific binding member, which in turn is. . .

DETD In addition, the **assays** can be performed in a variety of ways. Test sample, ASR, first specific binding member and **capture** reagent can be combined simultaneously in the **assay**, or they can be added and incubated individually or in combinations in a variety of sequences wherein the binding of. . . the reagents are combined as described in the following general and detailed examples should not be construed as limiting the **assay** method to that particular

order. It is preferable, however, not to react the ASR and the first specific binding member prior to adding test sample, because the **displacement** of the ASR from the first specific binding member by the sample analyte may require more time than does the. . .

DETD . . . the sample, then the analyte competes with the ASR for binding to the first specific binding member, or the analyte **displaces** the ASR from the first specific binding member. As a result, the remaining unbound or "free" ASR is proportional to. . .

DETD The mixture is then contacted to a **capture** reagent, e.g. a second specific binding member such as an anti-ligand antibody, immobilized upon a **solid** phase material. Some or all of the free ASR is immobilized upon the **solid** phase by the **capture** reagent which binds to the ligand-component of the ASR. The immobilized ASR can then be detected by adding an indicator. . . third specific binding member is capable of binding to the analyte-component of the ASR thereby forming a detectable or measurable **capture** reagent/ASR/indicator reagent complex on the **solid** phase. The third specific binding member can be identical to the first specific binding member, and therefore in this example,. .

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DETD In an alternative embodiment of the present invention, the **capture** reagent can be an analyte-specific binding member on the **solid** phase material, and the indicator reagent can be a labeled ligand-specific binding member. Other variations include, but are not limited to: the use of of multiple-layer **solid** phase devices wherein one or more of the necessary **assay** reagents are diffusively (i.e., capable of migrating through the **solid** phase) or non-diffusively (i.e., immobilized within or on the **solid** phase) incorporated in or on one or more of the layers; the use of teststrip materials for capillary, absorbent or chromatographic **assays** wherein one or more of the necessary **assay** reagents are diffusively or non-diffusively incorporated in or on the teststrip in one or more zones or sites; and the formation of **capture** reagent/ASR/indicator reagent complexes in solution, with detection of the complex either in solution or after the complex is separated from the solution by a **solid** phase. As known to those skilled in the art, the **solid** phase material can be designed to include a sufficient number of zones or layers, which contain the reagents necessary for the **assay**, so that the **assay** is substantially self-performing once a test sample is added.

DETD . . . is monovalent or too small to allow simultaneous binding to two specific binding members, its use is also advantageous in **assays** for larger analytes. For example, the present invention makes it unnecessary for an **assay** to involve two antibodies that are capable of binding to the same analyte without interfering with each other. Instead, only. . . of antibody recognition of overlapping epitopes and thereby facilitates the use of polyclonal as well as monoclonal antibodies in sandwich **assays**. Furthermore, because the same specific binding member may be used as the ligand-component of many different analyte/ligand combinations, the same ligand-specific binding member may be used as a generic reagent in many different **assays**. Therefore, a single indicator reagent (e.g., labeled anti-ligand antibody) or a single **solid** phase system comprising a **solid** phase material and an immobilized **capture** reagent (e.g., immobilized anti-ligand antibody) can be used without modification in many different **assays**, thereby increasing the ease of **assay** performance and decreasing the cost of device manufacture.

DETD In addition, an **assay** can be performed wherein a sandwich complex of **capture** reagent/ASR/indicator reagent is preformed and a first specific binding member is not used. This preformed complex can also be pre-attached to a **solid** phase material. This method is referred to as a reverse **assay** in which the analyte

(if present in the test sample) binds to the indicator reagent and **displaces** it from the **solid** phase thereby decreasing the signal associated with the **solid** phase at the site of immobilized **capture** reagent. This method also enables the performance of a multianalyte **assay**, which provides separate results for each analyte, using a "generic" **capture** reagent such as anti-fluorescein antibody positioned at three different sites on the **solid** phase. For example, a different sandwich complex can be preformed at each site with the generic **capture** reagent and (1) a fluorescein/tetrahydrocannabinol ASR and a labeled anti-tetrahydrocannabinol antibody indicator reagent, (2) a fluorescein/cocaine ASR and a labeled. . . a labeled anti-opiate antibody indicator reagent. A test sample containing one, two or all analytes can be contacted to the **solid** phase thereby displacing the respective indicators and demonstrating the presence of one or more analytes by decreasing signal production at. . .

DETD Multianalyte **assays** can also be performed by using appropriate ligand-components and analyte-components to form a different ASR to substitute for each different analyte in the **assay**, e.g.s, (1) a fluorescein/cocaine ASR, a (2) rhodamine/tetrahydrocannabinol ASR, and (3) an aminomethyl fluorescein/opiate ASR. A multianalyte **assay** providing separate results for each analyte can thereby be performed on a single **solid** phase using the appropriate **capture** and indicator reagents.

DETD **Assays** are also contemplated wherein a complex of first specific binding member/ASR/indicator reagent is preformed and reacted with the test sample, wherein analyte **displaces** ASR/indicator reagent subcomplex for subsequent reaction with a **capture** reagent. In a flow-through or test strip **assay** device for example, the first specific binding member/ASR/indicator reagent may be immobilized at a first reaction site and the **capture** reagent is immobilized at a second reaction site downstream from the first reaction site.

DETD The following examples illustrate methods for making the ASR of the present invention as well as methods for performing the **assay** procedures. The examples, however, are intended only to be illustrative and not limitative upon the scope of the invention, which. . . defined solely by the claims. It will be appreciated that one skilled in the art can conceive of many other **assays**, including semi-quantitative and quantitative **assays** to which the present inventive concepts can be applied.

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L8 ANSWER 6 OF 10 USPATFULL

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TITLE:	Magnetically assisted binding assays using magnetically labeled binding members
INVENTOR(S):	Rohr, Thomas E., Gurnee, IL, United States
PATENT ASSIGNEE(S):	Abbott Laboratories, Abbott Park, IL, United States (U.S. corporation)

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LEGAL REPRESENTATIVE:	Bach, Mark C.
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides devices for performing binding assays. Such devices comprise (i) a reaction vessel where unbound and immobilized magnetically-labeled reagent are produced in relation to the amount of said analyte in said test sample; (ii) a separation means for partitioning said immobilized magnetically-labeled reagent and said bound magnetically-labeled reagent; (iii) a magnetic field generator means for the application of a magnetic field to said magnetically-labeled reagent; and (iv) a measurement means to assess the effect of said magnetic field on said magnetically-labeled reagent as a measure of the presence or amount of said analyte in said test sample. The device provided by the instant invention can run, for example, direct indirect, competitive, inhibition and sandwich assay formats.

SUMM Diagnostic **assays** have become an indispensable means for detecting analytes in test samples by using the mutual reaction between the analyte and. . . tags or labels attached to antibodies, which in turn bind to the analyte of interest, are employed in such diagnostic **assays**, wherein the detection of the resultant labeled antibody/analyte complex, or of the labeled antibody which remains unbound, is used to. . .

SUMM Two commonly used binding **assay** techniques are the **radioimmunoassay** (RIA) and the enzyme **immunoassay** (EIA), both of which employ a labeled binding member. The RIA uses a radioactive isotope as the detectable tag or. . .

SUMM . . . may be difficult to purify and conjugate to binding members, and may be unstable during storage at room temperature. Enzyme **immunoassays** are also unsatisfactory in that the methods typically require complex incubations, multiple liquid additions and multiple wash steps. Moreover, even. . .

SUMM More recently, **assay** techniques using metallic sol particles as visual labels have been developed. In these techniques, a metal (e.g., gold, silver, platinum),. . . Generally, the binding member to be labeled is coated onto the metal sol particles by adsorption, and the particles are **captured** or aggregated in the presence of **analyte**. Although the metal sol particles have the advantage of producing a signal that is visually detectable as well as measurable. . . are difficult to quantitatively measure. The metallic particles also have a limited color intensity, and therefore limited sensitivity in

some **assays**. In addition, the **surfaces** of inorganic metallic colloid particles, such as gold, do not readily accept the covalent attachment of binding members. Thus, during use in a binding **assay**, care must be taken so that the adsorbed binding members are not removed from the inorganic particles through the combination of **displacement** by other proteins or **surface** active agents and the shear forces which accompany washing steps used to remove non-specifically bound material. Sol particles can be. . .

SUMM . . . have characteristics capable of affecting electrical resistance, wherein a capacitance measurement will reveal whether the particles are present on a **surface**. However, the effect of the magnetic field on the magnetic particles has no relation to the presence or amount of. . .

SUMM The present invention advantageously uses a magnetically-attractable material as a detectable label in binding **assays**. The magnetic label is subjected to a magnetic field and the label, in turn, displays a resultant force or movement. . .

SUMM . . . is selected to bind (i) the analyte or (ii) the first binding member, to thereby provide for competitive and sandwich **immunoassay** formats.

DETD The methods and devices of the present invention may be applied to any suitable **assay** format involving binding pair members including, but not limited to, those binding members described above. The **assay** methods of the present invention utilize the response of a magnetically-labeled reagent to the influence of a magnetic field to. . . to the present invention, the presence of an analyte mediates whether or not the magnetically-labeled reagent becomes immobilized to a **solid**-phase reagent. The analyte can thereby control the extent of the magnetically-labeled reagent's response to the influence of a magnetic field.. . .

DETD The present invention is applicable to various competitive **assay** formats and sandwich **assay** formats well known in the art. Various competitive, inhibition and sandwich **assays** have been described whereby a labeled reagent is partitioned between a liquid phase and a **solid** phase in relation to the presence of the analyte in the test sample. According to acompetitive **assay** format, a magnetically-labeled reagent can comprise a first binding member (an analyte analog) attached to a magnetically-attractable label, to thereby form a magnetically-labeled reagent. A **solid**-phase reagent can comprise a second binding member, whichis specific for the analyte and analyte analog, attached to a **solid** phase. During the course of the **assay**, an analyte in the test sample and the magnetically-labeled analyte analog compete for binding sites on the **solid**-phase reagent. Alternatively, the binding member attached to the **solid** phase may be an analyte-analog selected to compete with the analyte for binding to a magnetically-labeled binding pair member. Hence, the quantity of magnetically-labeled reagent that becomes bound to the **solid** phase is inversely proportional to the amount of analyte in the test sample.

DETD According to a sandwich **assay** format, a first binding member is attached toa magnetically-attractable label to form a magnetically-labeled reagent anda second binding member is attached to the **solid** phase to form a **solid**-phase reagent. The binding members are selected to directly or indirectly bind the analyte of interest. During the course of the **assay**, the magnetically-labeled reagent becomes immobilized to the **solid** -phase reagent by binding the analyte that has bound the **solid** -phase reagent. Thus, the quantity of magnetically-labeled reagent that becomes bound to the **solid**-phase reagent is directly proportional to the amount of analyte in the test sample.

DETD According to the present invention, **assay** protocols may optionally comprisethe use of ancillary binding members to indirectly bind the analyte or analyte analog to the magnetically-labeled reagent or to the **solid**-phase reagent. The ancillary binding member

can be attached to a **solid**-phase reagent or magnetically-labeled reagent before, during or after contactingthe **solid**-phase reagent or magnetically-labeled reagent with the test sample or other **assay** reagents. In addition, the **assay** protocols may comprise, for example, contacting the **assay** reagents and test sample simultaneously to form a reaction mixture, or the **assay** reagents and test sample can be contacted sequentially, and for a time period suitable for binding to form multiple reaction. . .

DETD According to such **assay** protocols, after a period suitable for binding, theunbound magnetically-labeled reagent can be separated from the bound magnetically-labeled reagent. It will. . . removal of the unbound magnetically-labeled reagent from the reaction mixture and/or from that magnetically-labeled reagent which is immobilized to the **solid**-phase reagent. The separation of bound and unbound magnetically-labeled reagent may also involve the segregation of the unbound magnetically-labeled reagent from. . .

DETD Generally, devices according to the present invention comprise components for performing magnetically assisted binding **assays** as taught herein. Accordingly, such devices preferably comprise (i) a reaction vessel; (ii) a separation means for separating the immobilized. . .

DETD The reaction vessel can be anything capable of containing the **assay** reagents disclosed herein and where unbound and immobilized magnetically-labeled reagent can be produced in relation to the amount of an analyte in a test sample. The reaction vessel can comprise any materialpreviously described herein with respect to the **solid** phase. Moreover, the **solid** phase or **solid**-phase reagent can serve as the reaction vessel such as, for example, test-tubes, microtiter wells, tubing, slides and the like.

DETD . . . or tilting means could be used to effectuate the separation. Preferably, the magnetically-labeled reagent that is not immobilized to the **solid**-phase reagent is separated from the **solid** phase by the application of a magnetic field which is sufficient to move unboundmagnetically-labeled reagent, but not the bound magnetically-labeled. . . wall, thereby pullingthe unbound magnetically-labeled reagent from the reaction mixture or away from the reagent which is immobilized to the **solid** phase. In yet another embodiment, a magnetic means may be brought into proximity with the **surface** of the reaction mixture such that unbound magnetically-labeled reagent is sequestered at the air/liquid interface of the reaction mixture, thereby. . .

DETD . . . including a resulting force or movement of the reagent such as, for example, an apparent weight change ofthe reagent, a **displacement** of the reagent, a mass change of the reagent, and the like. It will be understood, of course, that these. . . the magnetically-labeled reagent, or the manifestations can be measured indirectly by detecting the magnetically-labeled reagents effect on, for example, the **solid** phase, the **solid**-phase reagent or the magnetic field generator means. The influence of the magnetic field upon a magnetically-labeled reagent may be observed. . . mass can be detected and measured by a balance or a resultant change in frequency of a quartz crystal; a **displacement** can be detected and measured by an optical sensor means to assess the magnitude of a change from an initial position to a subsequent position assumed by the magnetically-labeled reagent, **solid**-phase reagent or **solid** phase; a movement can be detected and measured by motion detection means to assess movement, such as, for example, a. . . material; and a change in the amount of stresscan be detected by incorporating stress sensitive materials into a vessel or **solid** -phase material such that upon the application of a magnetic fieldthe change in stress would be detectable. It will be understood, of course,that depending upon the particular **assay**, it may be preferred to detect, directly or indirectly, the unbound

magnetically-labeled reagent's, the bound magnetically-labeled reagent's or both the. . .

DETD While various devices and **assay** protocols are contemplated by the present invention, the following protocols represent examples, and are not intended to be limited to, two sandwich **assay** formats using the magnetically assisted detection of a magnetically-labeled reagent of the present invention. In this regard, the following protocols,. . .

DETD 2) a second binding member selected to bind a second binding site on the analyte is attached to a **solid** phase to form a **solid** -phase reagent;

DETD . . . force upon the magnetically-labeled reagent immobilized on the bendable material. The force exerted upon the bendable material will cause a **displacement** of the material or a distortion in the shape of the material. The degree of **displacement** or distortion from the original position of the bendable material is largely dependent upon the amount of magnetically-labeled reagent bound to the **solid** phase and can be measured by the detection means.

DETD . . . reagent as a consequence of being exposed to magnetic field. Additionally, the Figures substantially correspond to Protocol B after the **solid** phase has been placed on the detection means (step 5). As seen in FIG. 5a, the **solid** phase comprises a well (10) that contains magnetically-labeled reagent (20), at least a portion of which isimmobilized in the well.. . . can be zeroed. In FIG. 5b, a magnet (60) ispositioned into or brought into proximity with the vicinity of the **surface** of the well contents whereby the magnetic field exerts a force upon the magnetically-labeled reagent. Under the influence of this. . . bound through the analyte to the well bottom. The unbound magnetically-labeled reagent at the air-liquid interface strains upward against the **surface** tension of the liquid **surface**, causing a change in the apparent weight of the **solid** phase which is registered as a change of the readout on the scale of the balance means (50). As the. . . the well, the increased intensity of the magnetic field results in a greater change in the apparent weight of the **solid** phase. As the magnetic field intensity increases, the weaker association of non-specifically bound magnetically-labeled reagent with the well bottom will. . .

DETD . . . to an automated operation or system. However, such automated operation or system is not meant to exclude the possibility thatsome **assay** operations in an automated system may be carried out manually.